

THE INFLUENCE OF CONSTANT AND CYCLIC ACCLIMATION TEMPERATURES ON THE METABOLIC RATES OF *PANOPEUS HERBSTII* AND *UCA PUGILATOR*<sup>1, 2</sup>

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Temperature is one of the major physical factors influencing the metabolic rates of intertidal invertebrates (Newell, 1975; Vernberg and Vernberg, 1972). Most previous studies on the respiratory metabolism of intertidal organisms have been conducted at constant temperatures and have utilized organisms acclimated to constant temperatures. Although these studies have led to many insights into the influence of temperature on respiratory adaptations, they may not describe the metabolic response of animals subjected to fluctuating thermal environments typical of those normally encountered in nature. Hence, a question can be raised concerning the value of the previous data from these studies at constant temperature in the analysis of the ecological energetics. To understand the significance of respiration in energy transfer in an ecosystem requires accurate estimates of oxygen consumption rates. This paper reports the results of a study on the comparative influence of constant and cyclic acclimation temperatures on the respiratory metabolism of two intertidal crabs which are common in South Carolina estuaries, the mud crab, *Panopeus herbstii* (Milne-Edwards), and the fiddler crab, *Uca pugilator* (Bosc).

Published data dealing with the influence of cyclic thermal environments on the physiology of marine invertebrate animals is limited, especially for respiratory metabolism. Earlier Kahn (1965) studied the effects of cyclic temperature on the growth of copepods while observations on larval crab growth under cyclic thermal regimes were reported by Costlow and Bookhout (1971), Christiansen and Costlow (1975), and Sastry and Vargo (1977). The influence of cyclic temperature on survival of crab larvae (Costlow and Bookhout, 1971; Sastry and Vargo, 1977) and of grass shrimp (Thorp and Hoss, 1975) has been reported. Sastry and Vargo (1977) recorded the metabolic response of larval crabs to cyclic temperatures, as did Humphreys (1975) for the nonmarine wolf spider. Widdows (1976) and Bayne, Widdows, and Worrall (1977) have reported on the influence of cyclic temperatures on the physiology of a bivalve, *Mytilus edulis*. Some investigators have reported that the metabolic response of marine animals is different depending on whether the temperature was increasing or decreasing (Van Winkle, 1969, for the mud snail, *Nassarius obsoleta*; Vernberg and Vernberg, 1966, for the fiddler crab, *Uca pugnax*).

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## MATERIALS AND METHODS

Mud crabs, *Panopeus herbstii*, were collected from intertidal oyster beds in the tidal creeks of the North Inlet Estuary near Georgetown, South Carolina. Fiddler crabs, *Uca pugnator*, were collected from nearby salt marshes where they are abundant on sand beaches. These species were selected because each has distinctive ecological requirements and both are present in great numbers. Thus, it is possible to compare responses of intertidal crabs from different habitats to determine any commonality of response by intertidal temperate zone animals.

The crabs were brought into the laboratory, washed in salt water, and placed in numbered partitioned plastic boxes containing 35‰ sea water. Then the boxes of crabs were kept in Revco Environmental Chambers in which light and temperature could be controlled. The crabs were fed every two days, and, after feeding, the crabs were placed in a clean box with fresh sea water. After seven days of acclimation to a constant temperature, the respiration rate of the crabs was determined using a Gilson Differential Respirometer. These results served as the baseline value against which measurements determined under fluctuating conditions were compared. Oxygen consumption was computed as  $\mu\text{l O}_2/(\text{hr} \cdot \text{g dry weight})$ , corrected to standard temperature and pressure.

After completing the initial metabolic determinations, the environmental chambers were programmed so that the animals would experience a once daily cyclic temperature regime where the previous constant acclimation temperature was the maximum temperature and the minimum temperature was 10° C. A daily 10° C thermal change was selected as this degree of fluctuation is not uncommonly experienced by these animals throughout much of the year. The change in temperature followed a square wave with about an hour of elapsed time before a new stabilized temperature was reached. After thermal cycling had started, respiration rates were measured on days 3, 6, 9, and 15–22. At the end of the experiments the crabs were dried in an oven at 105° C. Although different photoperiods have been shown to influence the metabolic response of crabs (Dehnel, 1958), the photoperiod regime was the same for animals exposed to both constant and fluctuating thermal experiments.

For simplicity of experimental design and to compare the relative effect of only thermal regimes, photoperiods were selected which corresponded to those the

TABLE I

*The various experimental conditions and number of organisms for each experiment.*

Cyclic temperature range (° C)	Photoperiod (L:D)	Constant acclimation temperature	Cyclic acclimation (days)	N
5–15 ( <i>Panopeus</i> )	8:16	15	3, 6, 9, 16	27
10–20 ( <i>Panopeus</i> )	12:12	20	3, 6, 9, 19	25
15–25 ( <i>Panopeus</i> )	14:10	25	3, 6, 9, 16	25
20–30 ( <i>Panopeus</i> )	14:10	30	3, 6, 9, 15	27
5–15 ( <i>Uca</i> )	8:16	15	3, 6, 9, 16	28
10–20 ( <i>Uca</i> )	12:12	20	3, 6, 9, 22	24
15–25 ( <i>Uca</i> )	14:10	25	3, 6, 9, 18	25
20–30 ( <i>Uca</i> )	14:10	30	3, 6, 9, 15	23

organisms typically experience at the different thermal ranges. For example, low temperatures normally occur during periods when the day length is short; while, in contrast, long days and high temperatures are usually coincident. A summary of the experimental conditions is given in Table I. The standard statistical techniques of Steel and Torrie (1960) were used to determine means, standard errors, and confidence intervals.

## RESULTS

*Panopeus herbstii* showed no statistically different change in its respiratory response at the low and high temperature ranges of 5–15° C and 20–30° C, with oxygen consumption rates the same after constant and cyclic acclimation (Fig. 1 and Table II). However, the oxygen consumption response to the middle acclimation temperatures (10–20° C and 15–25° C) varied with acclimation time. After 19 days exposure to cyclic temperature acclimation, the 10–20° C group was consuming about the same amount of oxygen as it did after acclimation to constant temperature. However, a statistically significant ( $P < 0.001$ ) decrease in metabolic rate occurred between day 6 and day 9 followed by a significant increase between day 9 and day 19. These responses resulted in a U-shaped metabolic curve. Animals exposed to the fluctuating temperature range of 15–25° C showed a significant metabolic decrease ( $P < 0.05$ ) by day 3 and another decrease ( $P < 0.01$ ) by day 6. The metabolic rate remained constant after this time until day 16 when the experiment was discontinued.

After exposure to three temperature ranges (5–15° C, 10–20° C, and 20–30° C) *Panopeus* had the same oxygen consumption rate before and after exposure to the cyclic thermal acclimation period. In contrast, those crabs exposed to 15–25° C had a significantly lower metabolic rate ( $P < 0.001$ ) than animals exposed to a constant temperature of 25° C (Fig. 2). Animals exposed to cyclic temperatures

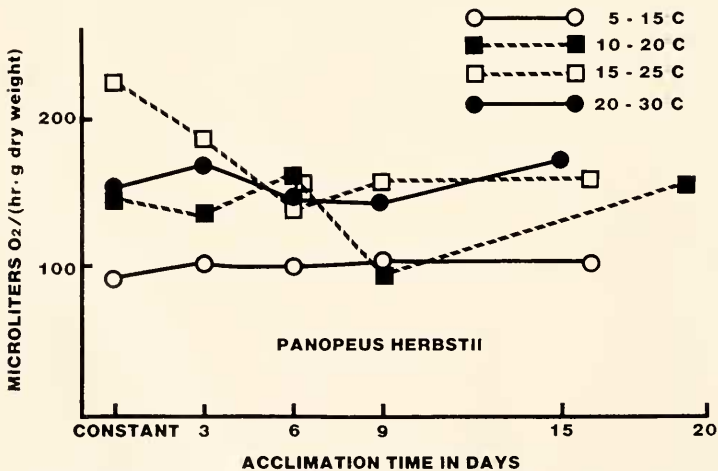


FIGURE 1. Oxygen consumption of *Panopeus herbstii* after constant acclimation (constant) and after varying lengths of time under the influence of cyclic temperatures.

TABLE II

Statistical analysis of metabolic-temperature responses of *Panopeus* and *Uca* exposed to fluctuating temperatures for different periods of time (NS indicates that means are not significantly different).

<i>Panopeus</i>				
Temperature range (° C)				
Days	5-15	10-20	15-25	20-30
0 vs. 3	NS	NS	$P < 0.05$	NS
3 vs. 6	NS	NS	$P < 0.01$	NS
6 vs. 9	NS	$P < 0.001$	NS	NS
9 vs. last	NS	$P < 0.001$	NS	NS

<i>Uca</i>				
Temperature range (° C)				
Days	5-15	10-20	15-25	20-30
0 vs. 3	NS	$P > 0.001$	NS	NS
3 vs. 6	NS	$P > 0.05$	NS	NS
6 vs. 9	$P > 0.01$	NS	NS	NS
9 vs. last	$P > 0.001$	NS	$P > 0.001$	$P > 0.05$

for 16-19 days exhibited an excellent degree of metabolic-temperature regulation in that the metabolic rate was the same at 20°, 25°, and 30° C. In contrast, when exposed to constant temperature, the metabolic-temperature curve showed an increase in metabolism with increasing temperature until a high stressful thermal

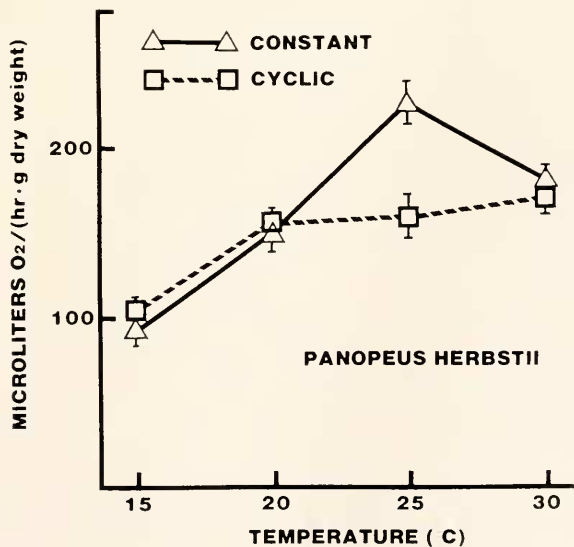


FIGURE 2. A comparison of the oxygen consumption of *Panopeus herbstii* after constant and cyclic acclimation temperatures. Vertical brackets are the standard errors of the means.

TABLE III

*Q*<sub>10</sub> values for metabolic rates of *Panopeus* and *Uca* exposed to constant and cyclic thermal regimes.

Thermal temperatures	Constant thermal regime		Cyclic thermal regime	
	<i>Panopeus</i>	<i>Uca</i>	<i>Panopeus</i>	<i>Uca</i>
15-20	2.6	1.5	2.3	1.2
15-25	2.5	1.1	1.6	1.2
20-25	2.3	1.0	1.1	1.2
20-30	1.1	1.2	1.1	1.5
25-30	<1.0	1.5	1.2	1.9

point (30° C) was reached and the rate decreased. *Panopeus herbstii* is killed at temperatures slightly over 30° C. These responses are better expressed as changes in *Q*<sub>10</sub> values (Table III), in that the *Q*<sub>10</sub> is less than one over the range of 25-30° C, but at all other temperature ranges the *Q*<sub>10</sub> is greater than one. The *Q*<sub>10</sub> values of animals maintained at constant temperature are higher than those of animals subjected to a cyclic thermal regime, except for those at thermal ranges involving 30° C. Of particular interest, animals exposed to a cyclic thermal regime did not show the same level of metabolic depression as did animals maintained at constant temperature. This response could have survival value to the *Panopeus* population.

The oxygen consumption of *Panopeus herbstii* with response to size and oxygen concentration has been investigated by Leffler (1973). This study utilized constant conditions, but a similar measurement technique. He showed that oxygen consumption was influenced by size following the general relationship expressed by von Bertalanffy (1957) and Hemmingsen (1960). Also, oxygen consumption in *P. herbstii* dropped in rough proportion to the oxygen level of the medium. Neither size nor oxygen concentration factors should have influenced our findings since the crabs were of approximately the same size (0.5-2.0 grams dry weight), and the crabs were partially exposed during the metabolic determinations in the Gilson respirometer. The oxygen consumption values reported by Leffler (1973) were of the same order of magnitude as those determined in our study, but exact conversion from Leffler's to our work is impossible since he used a lower salinity (22‰) than we (35‰). Recently Dimock and Groves (1975) have shown that the oxygen consumption of *Panopeus herbstii* is influenced by temperature and salinity combinations. Their observations of oxygen consumption are slightly lower than ours at the two most comparable temperature and salinity combinations (10° C and 30‰; 25° C and 30‰). The reduced metabolic values of Dimock and Groves' data are probably the result of using a full range of size classes, thus a larger average size being used in their studies.

The respiratory responses of *Uca pugnator* to increasing cyclic temperature acclimation time were different than those of *Panopeus* and were highly variable (Fig. 3). Interspecific differences were noted in that the fiddler crabs had the higher metabolic rate at each temperature. This result is consistent with the earlier finding of Vernberg (1969) that crabs which exhibit a high level of locomo-

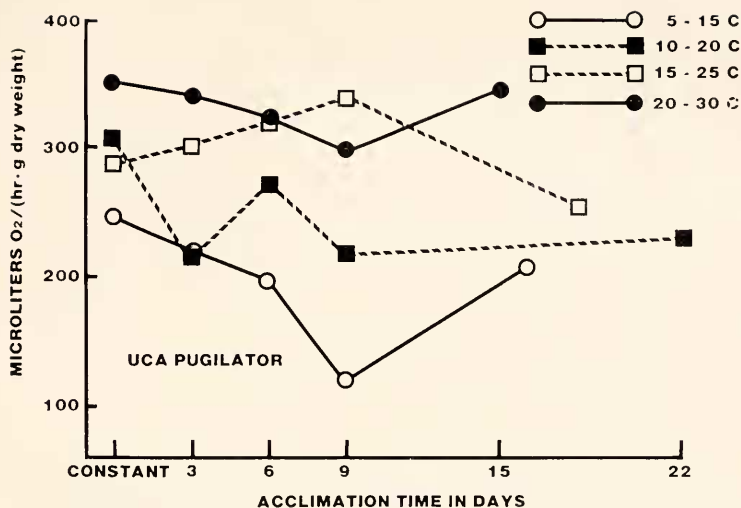


FIGURE 3. Oxygen consumption of *Uca pugnator* after constant acclimation (constant) and after varying lengths of time under the influence of cyclic temperatures.

tor activity tend to have a higher metabolic rate than a more lethargic species. *Uca* is an active crab at low tide when it can be seen darting about, while *Panopeus* is more secretive, hiding among the oyster shells. Also, some specimens of *Uca* are more temperature tolerant than *Panopeus*; an exposure to 30° C is less stressful on their metabolic response.

Following different exposure times to fluctuating temperatures, significant differences in means of metabolic rate were observed at each thermal regime (Table II). In contrast, significant differences occurred at only two of the four temperature ranges for *Panopeus*. However, when comparing the initial metabolic rate with the rate at the end of the exposure to fluctuating temperatures, only at 20° C was there a statistically significant difference with the rate being lower. After extended cyclic temperature acclimation, specimens of *Uca* acclimated to the warmest range (20–30° C) consumed significantly more oxygen than the other three groups which had similar rates (significant at the 95% confidence level). This trend suggests that specimens of *Uca* are less sensitive to thermal change than are those of *Panopeus*. Further evidence for this statement is that the  $Q_{10}$  values for *Uca* tend to be lower than those for *Panopeus* (Table III).

A graphic representation of the oxygen consumption rates of crabs maintained at constant and cyclic temperature is shown in Figure 4. The metabolic rate of *Uca pugnator* showed significant differences between constant- and cyclic-temperature-acclimated crabs at 20° C (95% level), 25° C (92% level), and 15° C (93% level), but not at the other temperature of 30° C. The metabolic response of *Panopeus* varied significantly only at 25° C. In the case of both species, the oxygen consumption rate was lower in animals subjected to cyclic temperatures.

The metabolic responses of *Uca pugnator* have been extensively investigated by Vernberg (1969). Constant-temperature-acclimated specimens of *U. pugnator* from the present study exhibited the same acclimation curve and metabolic rates as

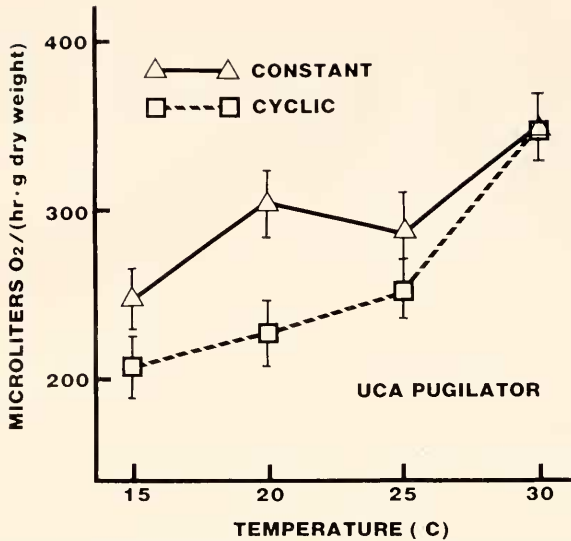


FIGURE 4. A comparison of the oxygen consumption of *Uca pugilator* after constant and cyclic acclimation temperatures. Vertical brackets are the standard errors of the means.

crabs from North Carolina. The cyclic-temperature-acclimated specimens of *U. pugilator* showed a depressed response compared to that of constant-temperature-acclimated crabs.

#### DISCUSSION

The experiments described here offer evidence that the metabolic rate of intertidal organisms, as measured by oxygen consumption, is influenced differently by constant and cyclic temperature acclimation regimes. The evidence is most striking in *Panopeus* exposed to the 15–25° C cycle and *Uca* exposed to 5–15°, 10–20°, and 15–25° C cycles. Other workers have demonstrated differences in various physiological response that could be correlated with variations in the type of thermal acclimation regime.

Thorp and Hoss (1975) determined that cyclic temperatures decreased survival of the grass shrimp, *Palaemonetes pugio* and *P. vulgaris*, at low salinities (5‰) and low cyclic temperatures (7–13° C) when compared to shrimp kept at constant temperatures under the same salinity. In contrast, the pupfish, *Cyprinodon nevadensis*, acclimated to cycling temperatures demonstrated a greater tolerance to both high and low temperature than animals acclimated to a constant temperature (Feldmeth, Stone, and Brown, 1974). This increased scope of thermal tolerance could have survival value to animals occupying habitats which characteristically are subjected to wide daily and seasonal thermal changes.

Cyclic temperatures may influence survival and development of larvae. Costlow and Bookhout (1971) found that survival of the larval mud crab, *Rhithropanopeus harrisi*, was about the same for a cycled temperature as for a constant temperature equal to the mean of the cycled temperatures over the range of 10–30° C, but

larvae maintained at a cycle of 30–35° C survived better than those at either 30° or 35° C. The time required to complete metamorphosis at the warmer cycles was influenced by the high temperature in the cycle at all salinities tested (Christiansen and Costlow, 1975). However, the results of these two studies at higher temperatures are different, possibly the result of variation in survival of crab larvae from hatch to hatch. More recently, Sastry and Vargø (1977) found that larvae of a decapod crustacean, *Cancer irroratus*, showed a greater survival rate when reared under a suitable amplitude and rate of temperature change than larvae maintained at comparable constant temperatures.

Cyclic temperature may influence sublethal responses of organisms, but not always in an apparent, predictable way. Hoffman (1974), working with crickets, reported that varying day-night temperature cycles do not accelerate physiological functions, except for life span and egg production.

Thorp and Hoss (1975) determined the oxygen consumption rate of two species of grass shrimp after acclimation to constant temperatures (7° and 10° C) and cyclic temperatures (7°–13° C) at salinities of 5, 20, and 35‰. Both species of shrimp utilized more oxygen when acclimated to constant temperature (10° C) and 35‰ than cyclic temperatures of 7–13° C and 35‰. This result is similar to our work in that our crabs were acclimated to 35‰, and when differences in oxygen consumption were observed, cyclic temperatures depressed oxygen consumption.

Unlike our results, the respiration rate of spiders maintained on a cyclic temperature regime was higher than animals kept at a constant temperature (Humphreys, 1975). This response may be correlated with an increase in growth and development rates of this species. However, since the resting metabolic rate is higher when kept on cyclic thermal regimes, the organisms must be more efficient in extracting energy from their food or they must eat more in order to grow. *Mytilus edulis*, a bivalve, utilizes a different strategy in adapting to cyclic temperatures. It reduces the amplitude of both oxygen consumption and filtration rate (Widdows, 1976). Specimens of *Panopeus* and *Uca* respond metabolically in a similar manner, at least at intermediate temperatures. One result of reducing the standard metabolic rate is to conserve energy which can be used for other functional activities necessary for an organism to successfully compete and survive. This is particularly important for these crabs in that the cyclic thermal ranges at which they demonstrate reduced rates of oxygen uptake are those in which these organisms are most active during most of the year.

Based on the results of this study it seems that any previous estimate of the role of oxygen consumption in energy budgets of a species and/or a community might be in error, unless the influence of cyclic temperature on respiration has been determined.

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## SUMMARY

The comparative influence of acclimation to constant and cyclic temperatures on the metabolic rates of the mud crab, *Panopeus herbstii*, and the fiddler crab, *Uca pugilator*, was observed. Although interspecific differences were observed, cyclic acclimation temperatures significantly depressed oxygen consumption in the 15°–25° C temperature range in both species when compared to rates of animals subjected to constant acclimation rates. Since this depression of metabolic rates occurs over that portion of the yearly temperature range within which the animals are most active, it is suggested that these organisms utilize energy more efficiently when subjected to natural cyclic temperature conditions than when subjected to constant temperature environments. This difference in metabolic data would be significant in analyzing the role of the yearly energy budgets of crabs in ecosystem energetics.

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